

Stoichiometry of the EF-Tu·GTP complex with aminoacyl-tRNA: ternary or quinternary?

Reuben Leberman*

European Molecular Biology Laboratory, Grenoble Outstation, BP 156, 38042 Grenoble Cedex, France

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Abstract The stoichiometry of the complex formed between the *Escherichia coli* polypeptide elongation factor EF-Tu, GTP and valyl-tRNA^{val} has been determined by non-enzymatic deacylation studies on mixtures of the components at well-defined concentrations. A titration end-point was found corresponding to a 1:1 complex of EF-Tu·GTP with the aminoacylated-tRNA i.e. formation of a ternary complex. The result conforms to the classical model of the elongation step and not to the revolutionary proposition of the formation of a 2:2:1 complex; quinternary complex (EF-Tu·GTP)₂·aa-tRNA.

Key words: Elongation factor Tu; Aminoacyl-tRNA; Deacylation; Ternary complex; Quinternary complex

1. Introduction

Four years ago a report was published [1] which proposed a radical modification to the accepted scheme for the elongation step in bacterial polypeptide synthesis. The report presented evidence that complexes formed between the polypeptide elongation factor, EF-Tu and aminoacylated elongator-tRNAs (aa-tRNAs) are not ternary complexes of EF-Tu·GTP·aa-tRNA but quinternary complexes of (EF-Tu·GTP)₂·aa-tRNA, and that the incorporation of one amino acid in a growing polypeptide was associated with the hydrolysis of two molecules of GTP. These claims challenge a scheme that has been established by a wide variety of experimental techniques over the past 20 years and triggered redeterminations of the stoichiometry of the complex between EF-Tu and aminoacylated-tRNA [2]. More recently, other evidence has been presented in support of the quinternary model [3]. Since previous data from this laboratory obtained by titration studies, measured by small angle neutron scattering, indicated the formation of a ternary complex [4], I have been prompted to redetermine the stoichiometry of the EF-Tu·GTP:aminoacyl-tRNA complex with particular attention to the measurement of the concentrations of the active components which is the crucial factor involved.

It has long been established that complex formation between EF-Tu·GTP and aminoacyl-tRNA protects the latter, leading to a decrease in the apparent non-enzymatic deacylation rate [5]. Since this decrease in the apparent deacylation rate is a function of the relative concentrations of aminoacyl-tRNA and EF-Tu·GTP, observation of this rate in the presence of varying, relative, initial concentrations of EF-Tu·GTP should provide information on the stoichiometry of the complex between aminoacyl-tRNA with EF-Tu·GTP.

2. Materials and methods

2.1. Labelled tRNA^{val}

Total *E. coli* (MRE 600) tRNA from Boehringer was aminoacylated using pure *E. coli* valyl-tRNA synthetase [6] with [¹⁴C]valine (Amersham) diluted with non-radioactive valine to an activity of 93 cpm/pmol. This was separated from excess radioactive amino acid by repeated precipitation with ethanol and resuspension in 0.01 M acetate buffer, pH 5, and the degree of valylation determined to be 73 pmol/A₂₆₀ by precipitation of samples on GF/A filters (Whatman) with 5% (w/v) trichloroacetic acid and processing the filters as previously described [7]. The [¹⁴C]valyl-tRNA was stored at –80°C until used.

2.2. EF-Tu·GTP

EF-Tu·GDP was isolated from *E. coli* (MRE 600) as described [8] with the modification of the use of Macro-Prep DEAE support (Bio-Rad) at the first chromatography step; EF-Tu·GDP is eluted later in the salt gradient and better separated from other protein components. EF-Tu·GTP was prepared by incubating an EF-Tu·GDP solution (at about 0.25 mM) for 3 h at room temperature with an equal volume of 10 mM GTP solution (Boehringer; special quality for molecular biology) containing in addition 10 mM PEP, 1 mg PK, 10 mM MgCl₂ and 10 mM KCl which had been preincubated for 30 min at room temperature. The reaction mix was then desalted on a PD 10 column (Pharmacia) pre-equilibrated in 100 mM triethanolamine-HCl, 50 mM NaOH, 1 mM PEP. The conversion of GDP to GTP was checked by HPLC.

2.3. Deacylation

In a total volume of 135 µl, 778 pmol of [¹⁴C]valyl-tRNA was reacted with samples of EF-Tu·GTP from 0 to 2022 pmol (based on [³H]GDP exchange) in 100 mM triethanolamine-HCl, 50 mM NaOH and 10 mM MgCl₂ at 37°C. After various time intervals, 25 µl samples were withdrawn and applied to GF/A filters pretreated with 100 µl of 5% trichloroacetic acid; the filters were then processed as described above for [¹⁴C]valyl-tRNA. The log₁₀ cpm was plotted vs. time to determine the apparent rates of deacylation. The deacylation curves shown are those obtained in the presence of varying amounts of EF-Tu·GTP.

3. Results and discussion

The present study used total *Escherichia coli* tRNA aminoacylated with only one amino acid so that the aminoacylated-tRNA has to compete against the non-aminoacylated-tRNAs for the EF-Tu·GTP and to use concentrations of tRNA that approximately correspond to those in vivo in *E. coli* [9]. Probably the most important aspects of the present determination are the direct measurements of the EF-Tu·GTP and aminoacyl-tRNA components by activity measurements; the former by first ascertaining that the all the nucleotide present in the sample is in the GTP form and then measuring the factor concentration by exchange with [³H]GDP [10], and the latter by direct labelling with [¹⁴C]amino acid.

Since the affinity of EF-Tu for GDP is about 100-times greater than for GTP it was deemed important to firstly verify the conversion of any GDP to GTP by the pyruvate kinase/phosphoenolpyruvate system, and then to remove the excess nucleotide to ensure that the concentration of any GDP

*Corresponding author.

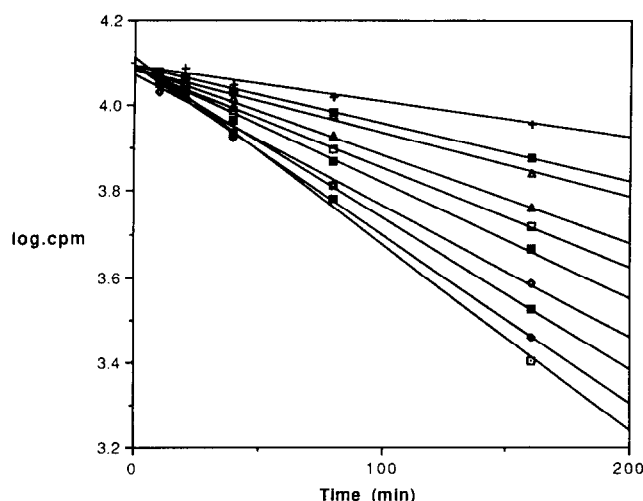


Fig. 1. Deacylation kinetics of 778 pmol [^{14}C]valyl-tRNA^{val} in the presence of, in ascending order, 0, 168, 337, 505, 674, 1011, 1348, 1685 and 2022 pmol EF-Tu·GTP.

produced by hydrolysis would be minimal. The other advantage of having only EF-Tu·GTP present is that the exchange for [^3H]GDP in the EF-Tu assay is rapid and complete due to the relative affinities for the two nucleotides.

Fig. 1 shows some of the data for the deacylation of [^{14}C]valyl tRNA, which is considered a pseudo first-order reaction, in the absence and presence of various amounts of EF-Tu·GTP. This shows, as expected, that complex formation leads to a decrease in the apparent rate of deacylation with increasing amounts of factor. Fig. 2 shows the relationship between these apparent rates ($\log_{10} \text{cpm}/\text{time}$) and the relative concentrations of EF-Tu·GTP/[^{14}C]valyl tRNA. At a ratio of 1:1 a breakpoint can be seen, indicating that there is a change in the degree of protection against non-enzymatic deacylation consistent with the formation of a ternary rather than a quaternary complex. This result is in total accord with the results of Bensch et al. [2] and with a myriad of other reports on these complexes. Furthermore, the complex between EF-Tu, a GTP analogue and an aminoacyl-tRNA that has been crystallized and is presently being analysed by X-ray diffraction, is of the ternary complex EF-Tu·GMPPNP·Phe-tRNA (J. Nyborg, personal communication).

It is important to emphasise that the formation of the EF-Tu:aminoacyl-tRNA complex is a separate issue to the number of GTP molecules hydrolysed per peptide bond formed. The latter appears to be clearly resolved by studies with the mutant EF-Tu which can bind XTP in place of GTP [3]. On the other hand, the stoichiometry of the complex is absolutely dependent on the correct determination of the active protein

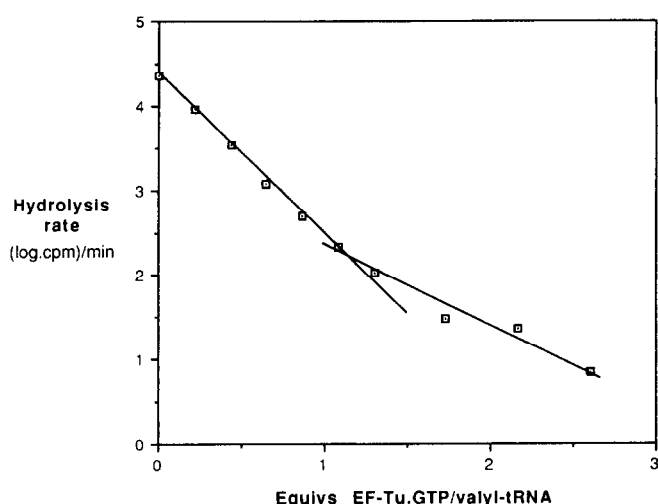


Fig. 2. The rates of the deacylation from Fig. 1 plotted against the ratios of EF-Tu:[^{14}C]valyl-tRNA^{val} in the reaction mixtures.

concentration which may be different to that measured by either spectroscopic or colorimetric methods. Thus, for example, measurements in this laboratory of EF-Tu·GDP by the Bradford [11] procedure standardized against bovine serum albumin led to values 2–3 times the concentration determined by nucleotide exchange.

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